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Mestrado em Biologia da Conservação

Dissertação orientada por:
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Resumo

Os anfíbios têm histórias de vida muito diversas e um ciclo de vida complexo, com características ecológicas diferentes em fases diferentes, desempenhando assim um papel importante nos ecossistemas. Acresce o seu grande contributo para a produção secundária e a biomassa dos ecossistemas. No entanto, as populações de anfíbios então em declínio à escala global e a uma velocidade alarmante. As principais ameaças conhecidas para as populações de anfíbios são as alterações do uso do solo, as alterações climáticas e surtos de doenças emergentes, como a quitridiomicose.

Os charcos são importantes para a reprodução de muitas espécies de anfíbios, e para o desenvolvimento dos ovos e das larvas, já que os ovos e as larvas de muitas espécies são aquáticos e os charcos são ricos em nutrientes e pobres em predadores. Os charcos são também ricos em biodiversidade, constituem elementos chave da paisagem, fornecem serviços de gestão da água, respostas às alterações climáticas e têm valor estético e recreativo. Muitos charcos foram construídos pelo homem com vista à sua utilização nas práticas agrícolas tradicionais. Por este motivo, a manutenção destes charcos artificiais é ameaçada pelas alterações do uso do solo, quer pela intensificação agrícola, quer pelo abandono.

Para compreender os factores que afectam a coexistência das espécies e a estrutura e a dinâmica das comunidades é necessário conhecer os padrões e os processos espaciais e temporais das comunidades. Adicionalmente, para compreender os fenómenos ecológicos, é necessário considerar as escalas espaciais e temporais em que estes ocorrem. Contudo a variação inter- e intra-anual destes fenómenos, como no caso da fenologia, apesar de ser reconhecida como tendo um papel importante na estrutura e no funcionamento dos ecossistemas, não tem sido convenientemente explorada.

Existem evidências de que alguns factores biofísicos podem influenciar a estrutura e a dinâmica das comunidades de anfíbios em charcos, nomeadamente a hidrologia, os factores climáticos, a área e a profundidade do charco, as características do habitat local, a altitude, a predação e a taxa inter-anual de substituição das espécies. Existem poucos estudos porém sobre a variação temporal das comunidades de anfíbios em charcos, e a maioria destes apenas aborda a variação inter-anual. A área do charco, a hidrologia, o clima, a predação, a dinâmica do habitat e factores regionais podem afectar a taxa inter-anual de substituição das espécies. No que diz respeito à variação intra-anual, existem algumas evidências de segregação temporal, algumas espécies têm dois períodos de reprodução, e muitas espécies exibem plasticidade no período de reprodução e no período larvar. Ademais, a temperatura e precipitação podem afectar a fenologia.

A região Mediterrânica é caracterizada por um clima altamente heterogéneo no tempo e no espaço, quer em escalas locais quer regionais, resultando numa elevada imprevisibilidade temporal e espacial das condições ambientais. Esta imprevisibilidade pode influenciar a ecologia e o comportamento reprodutores das espécies: por exemplo, as espécies podem exibir plasticidade quanto ao início da reprodução e na duração do período larvar; e a selecção de habitat de reprodução pode mudar de acordo com a hidrologia.

São raros os estudos que abordam a variação intra-anual de toda a comunidade de anfíbios em múltiplos charcos. Neste trabalho investigámos e modelámos a variação intra-anual de comunidades de larvas de anfíbios em charcos numa paisagem Mediterrânica de transição, no Sudoeste da Península Ibérica. Este estudo tem dois objectivos: (1) detectar padrões intra-anuais na estrutura das comunidades e na fenologia das espécies, e (2) investigar a influência de factores climáticos (precipitação e temperatura) e da hidrologia (profundidade do charco) na estrutura das comunidades e na abundância específica.

A área de estudo situa-se no Parque Natural da Serra de São Mamede e no Sítio de Importância Comunitária da Rede Natura 2000 de São Mamede. Esta região inclui a serra mais alta a

sul do rio Tejo, em Portugal, alcançando 1025 m e tendo orientação NW-SE. O clima da região é Mediterrânico de influência Atlântica. A cadeia montanhosa de São Mamede tem uma elevada diversidade de fauna e flora, sustentada pela grande variedade climática e pela grande diversidade de habitats. Esta região é altamente adequada para o estudo da estrutura e da dinâmica de comunidades de anfíbios, e das interações e da persistência das espécies, pois a diversidade de anfíbios é elevada, sendo uma das mais altas na Península Ibérica. Os charcos estudados eram temporários, com hidroperíodo longo (apenas um charco secou anualmente) e hidrologia diversa.

Recolhemos dados sobre a presença e a abundância de larvas e de ovos de anfíbios em oito charcos durante dois anos e meio. Amostrámos as larvas e os ovos de anfíbios através de arrastos com camaroeiro. Fizemos prospecções por contacto visual para complementar a informação sobre a presença de larvas e de ovos das diferentes espécies. Os dados climáticos foram obtidos da estação meterológica mais próxima.

Analisámos o padrão temporal (variação mensal) da estrutura das comunidades ajustando um modelo periódico sinusoidal, baseado num ciclo de actividade sazonal circa-anual, à riqueza específica, à diversidade de larvas e de ovos, e à abundância total de larvas e de ovos. A fenologia de cada espécie foi analisada através do número de charcos ocupados (por larvas e por ovos) em cada mês. As relações entre a fenologia das comunidade e espécies e as variáveis climáticas (temperatura e precipitação) e hidrológicas (profundidade do charco) foram analisadas utilizando regressão linear múltipla.

Os nossos resultados apoiam a existência de um padrão intra-anual na estrutura das comunidades de larvas de anfíbios dos charcos. A riqueza específica, a diversidade de larvas e de ovos, e a abundância total de larvas e de ovos revelaram um padrão sazonal circa-anual, com um pico na primavera. O modelo sinusoidal ajustado apoia a existência deste padrão.

No nosso estudo cada espécie de anfíbio exibiu um padrão fenológico coerente, e estes padrões variaram entre espécies. O padrão sazonal observado na estrutura das comunidades resulta da combinação destes padrões fenológicos de cada espécie.

Como esperado, observou-se um desfasamento entre as curvas sinusoidais da diversidade de ovos e de larvas, com a curva dos ovos precedendo a das larvas, sucedendo o mesmo para a abundância total. Contudo o desfasamento foi menor para a diversidade do que para a abundância.

Adicionalmente, a fenologia das espécies também revelou alguma variabilidade, entre anos e entre charcos. A plasticidade fenológica das espécies pode favorecer a persistência das comunidades em ambientes imprevisíveis como a região Mediterrânica, com a sua característica heterogeneidade climática no tempo e no espaço.

Os nossos resultados sugerem que a segregação temporal dos períodos de reprodução e de desenvolvimento larvar pode contribuir para a coexistência de espécies, e assim para a persistência de comunidades diversas.

A resposta das espécies e das comunidades aos factores ambientais (temperatura, precipitação e profundidade do charco) foi largamente coerente com o conhecimento anterior sobre a sua ecologia. Os nossos resultados apoiam a importância de charcos com um hidroperíodo longo, juntamente com a manutenção de uma rede de charcos com uma hidrologia variável de acordo com causas naturais, para a conservação da diversidade de anfíbios na região Mediterrânica.

Adicionalmente, porque a riqueza e a diversidade específicas, bem como a fenologia de muitas espécies, são afectadas pelo clima e pela hidrologia, as alterações climáticas e a manipulação da hidrologia dos charcos, nomeadamente a sua regularização e o seu nivelamento, podem influenciar a estrutura e persistência das comunidades de anfíbios.

Este estudo estende a aplicação de modelos sinusoidais a comunidades de larvas de anfíbios. Estes modelos permitem a previsão de tendências futuras na dinâmica das comunidades, o que pode

contribuir para melhorar as práticas de gestão para a conservação. A pesquisa futura deverá focar-se no desenvolvimento destes modelos e no seu acoplamento com a modelação de variáveis ambientais.

Por fim, a presença de variação intra-anual sugere que os estudos sobre a diversidade de anfíbios devem cobrir o ano inteiro. Adicionalmente, a variabilidade inter-anual observada na região de estudo, assim como as grandes flutuações temporais documentadas para outras populações de anfíbios, indicam que estudos a longo prazo são necessários para compreendermos plenamente os padrões e os processos destas comunidades.

Palavras-chave
Charcos temporários; fenologia; modelo sinusoidal; padrões temporais; variação intra-anual.

Abstract

We investigated and modeled the intra-annual variation of amphibian larvae communities in ponds in a Mediterranean transitional landscape, in the southwest of the Iberian Peninsula. This study has two goals: (1) to detect intra-annual patterns in community structure and species phenology, and (2) to investigate the influence of climate (precipitation and temperature) and hydrology (pond depth) on community structure and species abundance. We collected data on presence and abundance of amphibian larvae and eggs in eight ponds over a period of two and a half years. Our findings support the existence of an intra-annual pattern in amphibian larvae pond communities. Species richness, diversity of larvae and eggs, and overall abundance of larvae and eggs showed a seasonal pattern, with a peak in the spring, as supported by the fitted sinusoidal model. Each amphibian species exhibited a coherent phenological pattern, and patterns varied among species. The seasonal pattern of the amphibian community structure resulted from the combination of the phenological patterns of the different species. Some phenological variability was also detected in all species. Phenology plasticity might favor the persistence of diverse communities in unpredictable environments as the Mediterranean region. Our results also suggest that temporal segregation of the breeding and larval periods may contribute to species coexistence and thus to the persistence of diverse communities. The effects of environmental factors were consistent with previous knowledge on the species and the communities ecology. Climate and hydrology affect species richness, diversity, and phenology, therefore climate change and manipulation of pond hydrology may influence community structure and persistence. This study expands the application of sinusoidal models to amphibian larvae communities. These models can be used to predict future trends in community dynamics, which can contribute to improve conservation management practices. Finally, the occurrence of intra-annual variation suggests that amphibian surveys need to cover the entire year. Moreover, the inter-annual variability observed in this and other studies indicate that long term studies are necessary to better understand these community patterns and processes.

Keywords
Intra-annual variation; phenology; sinusoidal model; temporal patterns; temporary ponds.

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List of Abbreviations

AICc	Corrected Akaike Information Criterion
a.s.l.	Above sea level
CPUE	Catch-per-unit-effort

List of Symbols

y	Dependent variable
α	Mean level of dependent variable
A	Amplitude
f	Frequency
t	Time
ϕ	Phase
ε	Error
S	Mean species richness
D_L	Mean diversity of larvae
D_E	Mean diversity of eggs
Ab_L	Mean overall abundance of larvae
Ab_E	Mean overall abundance of eggs
M	Month

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Introduction

Amphibians have diverse life histories and a complex life cycle with distinct ecological traits, and play an important role in ecosystems, contributing greatly to secondary production and biomass (Duellman & Trueb, 1994; Morand, 2001; Wells, 2007). However, amphibians are declining at an alarming rate on a global scale. The known major threats are land use change, climate change, and outbreaks of emerging diseases, such as chytridiomycosis (Houlahan et al., 2000; Stuart et al., 2004; Pereira et al., 2010; Hof et al., 2011).

The knowledge of species ecology and community structure and dynamics fosters the comprehension of biodiversity patterns and processes (Pereira et al., 2010, 2013). To understand the factors affecting species coexistence and community assembly rules, knowledge of spatial and temporal patterns and of processes of community structure is needed (Rosenzweig, 1995; Odum & Barrett, 2005; Morin, 2011). The processes affecting community structure changes over time are colonization, extinction, migration, skipped breeding, and phenology (Duellman & Trueb, 1994; Rosenzweig, 1995; Wells, 2007; Morin, 2011). Additionally, in order to understand ecological phenomena, the spatial and temporal scales at which they occur need to be considered (Rosenzweig, 1995; Morin, 2011). Nevertheless, inter- and intra-annual variation, such as in phenology, despite being recognized as playing a role in ecosystems structure and functioning, have not been well explored (Rosenzweig, 1995; Morin, 2011).

Small water bodies are linked to amphibian ontogeny and recruitment, since the eggs and larvae of most species are aquatic (Duellman & Trueb, 1994; Wells, 2007). Thus, ponds are important for many amphibian species reproduction, and for egg and larval development, as they are rich in nutrients and poor in predators (Griffiths, 1997; Morand, 2001; Grillas et al., 2004; Oertli et al., 2005; Ruiz, 2008; Williams et al., 2010).

Ponds have high biodiversity, are key landscape elements, provide water management services, enable responses to climate change, and have aesthetic and recreational value (Grillas et al., 2004; Oertli et al., 2005; Ruiz, 2008; Williams et al., 2010; Céréghino et al., 2014). Many ponds are man-made and used in traditional agricultural practices, and thus their persistence is threatened by land use change, either through agricultural intensification or abandonment (Grillas et al., 2004; Oertli et al., 2005; Ruiz, 2008; Williams et al., 2010).

There is evidence that some biophysical factors can influence amphibian pond community structure and dynamics, namely hydrology, climate factors, pond area and depth, local habitat characteristics, altitude, predation, and species inter-annual turnover (Oertli et al., 2002; Beja & Alcazar, 2003; Jakob et al., 2003; Richter-Boix et al., 2006; Werner et al., 2007; Vignoli et al., 2007; Both et al., 2009; Gómez-Rodríguez et al., 2010a, 2010b; De Marco et al., 2014; Jeliazkov et al., 2014). However, there are few studies on temporal variation of amphibian pond communities, and most of them only address inter-annual variation. Pond area, hydrology, meteorology, predation, habitat dynamics, and regional factors can affect species inter-annual turnover (Werner et al., 2007; Gómez-Rodríguez et al., 2010a, 2010b). Concerning intra-annual variation, there is some evidence that there is temporal segregation, that some species have two breeding periods, and that many species exhibit plasticity in the breeding and larval periods. Furthermore, temperature and rainfall can affect phenology (Díaz-Paniagua, 1988, 1992; Jakob et al., 2003; Richter-Boix et al., 2006; Vignoli et al., 2007; Both et al., 2009).

Most knowledge on amphibian community patterns and processes comes from tropical regions and temperate regions of North America and Europe (see Wells, 2007), but less is known for the Mediterranean region. The Mediterranean region is characterized by a highly heterogeneous climate in time and space, from local to regional scales, which results in high temporal and spatial unpredictability of the environmental conditions (Blondel & Aronson, 1999). This unpredictability may influence species reproductive ecology and behavior; in particular, species can show plasticity in the onset of breeding and in the duration of the larval development (Díaz-Paniagua, 1988, 1992; Jakob et al., 2003; Richter-Boix et al., 2006); and breeding habitat selection can change according to hydrology (Beja & Alcazar, 2003; Jakob et al., 2003; Vignoli et al., 2007; Gómez-Rodríguez et al., 2010a, 2010b; Ferreira & Beja, 2013).

Studies addressing the intra-annual variation of the whole amphibian species assemblage in multiple ponds are rare (Díaz-Paniagua, 1988, 1992; Jakob et al., 2003; Vignoli et al., 2007). Here we investigated the intra-annual variation of amphibian larvae communities in ponds in a Mediterranean transitional landscape, in the southwest of the Iberian Peninsula. The main objective of our study was to model the phenology of these communities. The study can be broken down into two goals: (1) to detect intra-annual patterns in community structure and species phenology, and (2) to investigate the influence of climate factors (precipitation and temperature) and hydrology (pond depth) on community structure and species abundance.

Materials and methods

Study area

The study area was Serra de São Mamede Natural Park and São Mamede Natura 2000 Site of Community Importance (Fig. 1). São Mamede mountain range has high faunal and floral diversity, sustained by great diversity of climates and habitats (Pargana et al., 1996; I.C.N., 2006). This region is highly suitable for studying amphibian community structure and dynamics, and species interactions and persistence, as amphibian diversity is high, one of the highest in the Iberian Peninsula (Pargana et al., 1996).

São Mamede Mountain is the highest mountain south of the river Tagus in Portugal, reaching up to 1025 m a.s.l., and having a NW-SE direction. Its climate is Mediterranean with Atlantic influence (Pargana et al., 1996). The main land covers are open oak woodland (of *Quercus suber* L. and *Quercus ilex* L.), eucalypt (*Eucalyptus globulus* Labill.) and pine (*Pinus pinaster* Aiton) plantations, oak forest (of *Quercus pyrenaica* Willd.), agriculture, pasture, scrubland, and urban (I.C.N., 2006). This region encompasses the southern limit of several species ranges, including two amphibians: *Alytes obstetricans* (Laurenti, 1768) and *Rana iberica* Boulenger, 1879 (Pargana et al., 1996). Another nine anurans [*Alytes cisternasii* Boscá, 1879; *Discoglossus galganoi* Capula, Nascetti, Lanza, Bullini & Crespo, 1985; *Pelobates cultripes* (Cuvier, 1829); *Pelodytes punctatus* (Daudin, 1802); *Bufo bufo* (Linnaeus, 1758); *Epidalea calamita* (Laurenti, 1768); *Hyla arborea* (Linnaeus, 1758); *Hyla meridionalis* Boettger, 1874; and *Pelophylax perezi* (López-Seoane, 1885)] and four urodeles [*Pleurodeles waltl* Michaelles, 1830; *Salamandra salamandra* (Linnaeus, 1758); *Lissotriton boscai* (Lataste, 1879); and *Triturus pygmaeus* (Wolterstorff, 1905)] are present in the region (Loureiro et al., 2008).

We did a preliminary survey of the water bodies in the study area in order to select eight ponds based on biophysical criteria (Fig. 1). The ponds were temporary, with long hydroperiod (only one pond dried annually), and diverse hydrology. The maximum inundation area of the ponds ranged from 100 to 1500 m^2, and pond altitude ranged from 300 to 700 m a.s.l. Ponds were surrounded by

open oak woodland (*Q. suber*), pine (*P. pinaster*), oak (*Q. pyrenaica*), and pasture, and were used for livestock watering. One pond was leveled in August 2002, remaining as a small temporary pond.

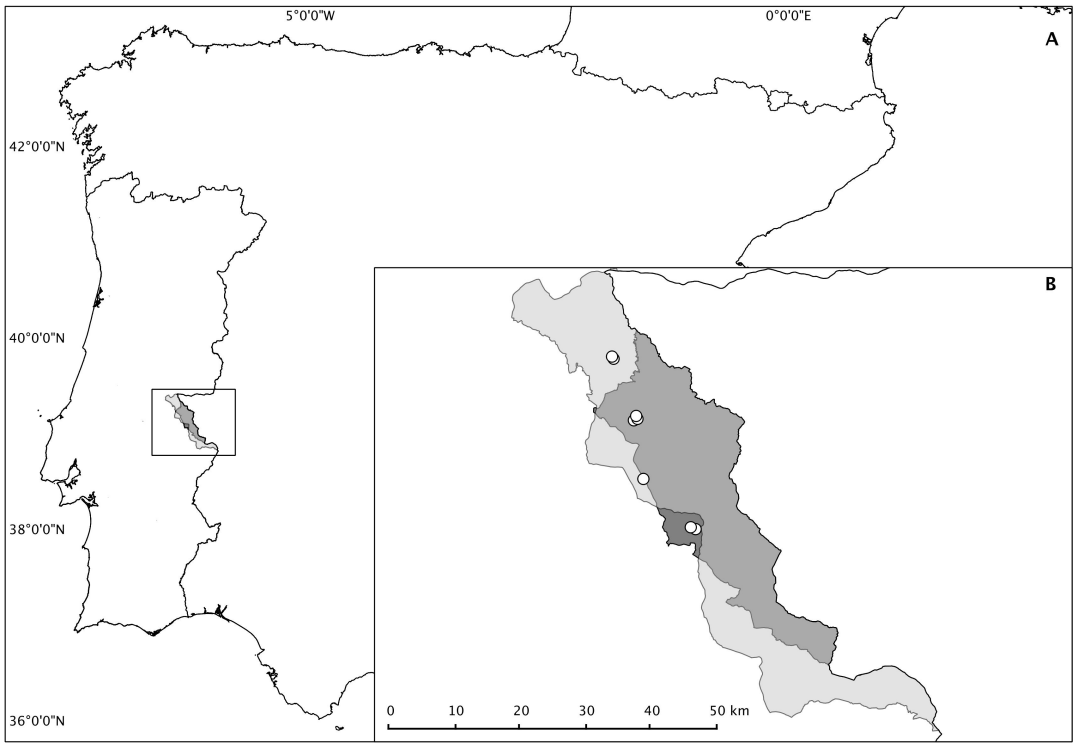

Figure 1. A. Location of the study area in the Iberian Peninsula. B. Map of the study area. White circles: ponds; dark grey: Serra de São Mamede Natural Park; light grey: São Mamede Natura 2000 Site of Community Importance.

Sampling methods

We sampled the ponds from the spring of 2001 to the summer of 2003: monthly in the spring, twice in the summer, once in the autumn, and once in the winter. Exceptions were the spring and the summer of 2001, when ponds were sampled once, and the autumn of 2001, when they were sampled twice. Some ponds were not sampled in some months due to logistical constraints (n = 13 events). We sampled amphibian larvae and eggs through dip netting. We used 1 mm mesh dip nets with 20 cm diameter frame, and the same person swept 2 m wide, from 8 to 12 times, depending on pond size, covering the existing microhabitats (Heyer et al., 1994). Collected specimens were returned to the ponds of origin immediately after sampling and identification. We used visual surveys to complement the information on the presence of species larvae and eggs. In each sampling event (pond and month) we registered the pond characteristics. Climate data (temperature and precipitation) were obtained from the nearest meteorological station at "Campo Experimental do Crato (Chança)". Climate data were not available for June 2001, February 2002, and August 2002.

Data analysis

For each species and each sampling event (pond and month), abundance was expressed as catch-per-unit-effort (CPUE), that is, the mean number of individuals (larvae or eggs) per 1 m sweep. We used egg CPUE instead of presence because we were interested in analyzing a measure of abundance. Different species lay their eggs either in masses, strings, or separated but near each other, and thus it is probable that a sweep catches several eggs from the same clutch. However, egg clutches have very different sizes, and this would be reflected in the abundance measure. We computed pond overall abundance in each month as the sum of the CPUE of all species. Abundance was log transformed [log (CPUE + 1)]. We calculated species diversity using Simpson's index (Magurran, 1988). Abundance and diversity were analyzed separately for larvae and for eggs. Mean monthly pond maximum depth (cm) was computed as the mean of the maximum pond depths for each month. *Bufo bufo* and *E. calamita* larvae and *P. cultripes* eggs were excluded from the analyses due to the reduced number of observations.

We analyzed the temporal pattern (monthly variation) of community structure by fitting a periodic model based on a circa-annual seasonal cycle of activity to the species richness, the diversity of larvae and of eggs, and the overall abundance of larvae and of eggs (Mean ± SE) (Shumway & Stoffer, 2006; Crawley, 2007). Specifically, we applied to each of these variables the following sinusoidal model

$$y = \alpha + A\sin(2\pi ft + \phi) + \varepsilon \qquad (1)$$

where α is the mean level of y, A is the amplitude, f is the frequency, t is the time, ϕ is the phase, and ε is the error. We fitted non-linear least squares models (Crawley, 2007) to the mean species richness, the mean species diversity, and the mean overall abundance, assuming a 12-month period, and month as the time variable. Parameter significance was assessed with t tests. The larvae abundance in October 2002 was identified as an outlier and excluded from this analysis: this extreme value reflects the collection of many larvae of *P. cultripes*, an explosive breeder with large clutches (Arnold & Ovenden, 2002; García-París et al., 2004).

The phenology of each species was investigated by means of the number of ponds occupied (larvae and eggs) per month. When a pond was not sampled in a month and a species was present in the previous and the subsequent months, we considered it was present that month (n = 6 cases).

We investigated the relationship between phenology, climate variables (temperature and precipitation), and hydrology using multiple linear regression (Crawley, 2007). Response variables were, at the assemblage level, mean monthly species richness, mean monthly species diversity, and mean monthly overall abundance, and at the species level mean monthly abundance. Predictor variables were total monthly precipitation (mm), mean monthly temperature (°C), and mean monthly pond maximum depth (cm). Initially we also considered sun hours, however, due to high collinearity with temperature (Spearman's correlation test, $r = 0.756$, $P < 0.001$) and precipitation (Spearman's correlation test, $r = -0.879$, $P < 0.001$), this predictor variable was removed from the analysis (Shaw, 2003). Model selection was based on the corrected Akaike Information Criterion (AICc) (Crawley, 2007). Significance of the predictors was assessed through t tests (Crawley, 2007).

The alpha-level for significance was set at $P < 0.05$. Statistical analyses were performed using the R statistical software (R Development Core Team, 2011) and the packages Vegan (Oksanen et al., 2013) and AICcmodavg (Mazerolle, 2013).

Results

During this study we observed all the fifteen amphibian species present in the study area (Loureiro et al., 2008). However, the eggs and the larvae of *D. galganoi*, *P. punctatus*, and *R. iberica* were not recorded in the ponds. In addition, the eggs of *B. bufo* and *E. calamita* were not collected and the larvae of these species were only rarely collected. The eggs of *S. salamandra*, *A. cisternasii,* and *A. obstetricans* are not found in water bodies due to the life history characteristics of these species (García-París et al., 2004). Finally, the eggs of *P. cultripes* were rarely observed.

We collected 3236 larvae (1552 from four urodeles species and 1684 from eight anuran species) and 1979 eggs (687 from three urodeles species and 1292 from three anuran species). Species richness per pond per month ranged from zero (no species) to seven species, the diversity (Simpson's index) of larvae ranged from zero to 0.829 and of eggs from zero to 0.656, and the overall abundance (CPUE) of larvae ranged from zero to 12.35 and of eggs from zero to 20.00.

Communities showed a circa-annual pattern (Fig. 2, Table 1). Species richness and diversity of larvae and eggs showed a peak in spring, as did larvae and egg overall abundance. However, high larvae abundance was recorded in October 2002, due to the collection of a great number of larvae of *P. cultripes*. This circa-annual pattern was supported by the sinusoidal model fitted to the mean species richness ($R^2 = 0.65$, Table 1), to the mean larvae diversity ($R^2 = 0.69$), and, to a lesser extent, to the mean egg diversity ($R^2 = 0.50$), to the mean larvae overall abundance ($R^2 = 0.38$), and to the mean egg overall abundance ($R^2 = 0.53$).

Additionally, there was a time lag between the sinusoidal waves of egg and larvae diversity, as well as for overall abundance. However, note that the phase shift for the larvae overall abundance is not statistically significant (Table 1). The wave of eggs led that of larvae in both cases, though with a smaller time interval for diversity than for overall abundance.

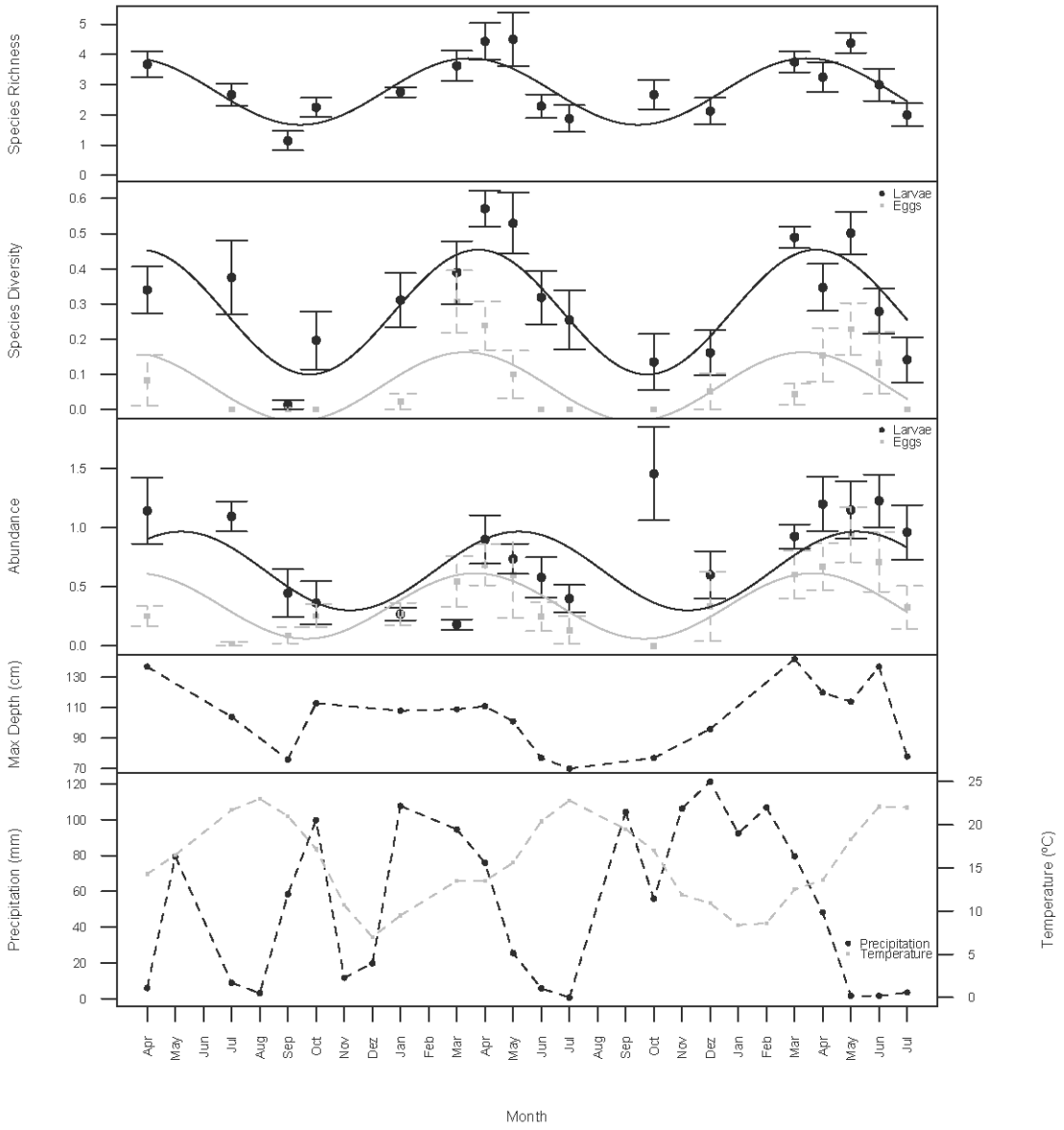

Figure 2. Species richness (Mean ± SE), diversity of larvae and eggs (Simpson's index, Mean ± SE), overall abundance of larvae and eggs [log (CPUE + 1), Mean ± SE], and environmental variables (mean monthly pond maximum depth (cm), total monthly precipitation (mm), and mean monthly temperature (°C)), from spring 2001 to summer 2003. Sinusoidal models (full lines) fitted to mean species richness (S), mean diversity of larvae (D_L) and eggs (D_E), and mean overall abundance of larvae (Ab_L) and eggs (Ab_E), where M is the month (see Table 1): $S = 2.771 + 1.101\sin(2\pi\, M/12 + 1.341)$; $D_L = 0.277 + 0.177\sin(2\pi\, M/12 + 1.171)$; $D_E = 0.065 + 0.098\sin(2\pi\, M/12 + 1.410)$; $Ab_L = 0.635 + 0.333\sin(2\pi\, M/12 + 0.415)$; $Ab_E = 0.338 + 0.277\sin(2\pi\, M/12 + 1.232)$.

Table 1. Sinusoidal models relating mean species richness, mean diversity (Simpson's index) of larvae and eggs, and mean overall abundance [log (CPUE + 1)] of larvae and eggs to month as the time variable. Non-linear least squares models parameters (α: mean level, A: amplitude, ϕ: phase) and t tests results.

Dependent variable	R^2	Parameter	Estimate	SE	t	P
Species richness	0.65	α	2.771	0.165	16.798	< 0.001
		A	1.101	0.214	5.135	< 0.001
		ϕ	1.341	0.213	6.310	< 0.001
Diversity Larvae	0.69	α	0.277	0.024	11.326	< 0.001
		A	0.177	0.031	5.631	< 0.001
		ϕ	1.171	0.197	5.937	< 0.001
Diversity Eggs	0.50	α	0.065	0.020	3.239	0.006
		A	0.098	0.026	3.737	0.002
		ϕ	1.410	0.289	4.876	< 0.001
Abundance Larvae	0.38	α	0.635	0.088	7.207	< 0.001
		A	0.333	0.118	2.831	0.014
		ϕ	0.415	0.344	1.208	0.249
Abundance Eggs	0.53	α	0.338	0.054	6.278	< 0.001
		A	0.277	0.069	3.983	0.001
		ϕ	1.232	0.277	4.440	< 0.001

Each species showed a coherent phenological pattern among years and across ponds (Fig. 3). In addition, breeding dates and larval periods varied among species. Excluding rare occurrences, two species were present during the whole year (*L. boscai* and *P. perezi*), four species were present from autumn to spring (*P. waltl*, *S. salamandra*, *A. cisternasii*, and *P. cultripes*), one species was present in spring and summer (*T. pygmaeus*), and two species were present only in spring (*H. arborea* and *H. meridionalis*). *Alytes obstetricans* larvae were recorded only six times at one pond, from spring to summer (twice in 2001, and thus are not presented in Fig. 3), and once at another pond, in autumn. Moreover, *L. boscai* and *P. perezi* had overwintering larvae. Finally, *A. cisternasii* and *A. obstetricans* had two breeding periods, autumn and spring/summer.

Nevertheless, to different degrees, all species presented variability in their phenology between years: seven species had different months of occurrence between years, and all species presented differences concerning occupied ponds and number of occupied ponds in the same month in different years. Additionally, all species showed inter-pond variability: all species presented differences in phenology between ponds, eight species were not recorded at least in one pond, three species were recorded in less than five ponds per year (*A. cisternasii*, *A. obstetricans,* and *H. arborea*), and only one species (*T. pygmaeus*) was recorded in all ponds each year.

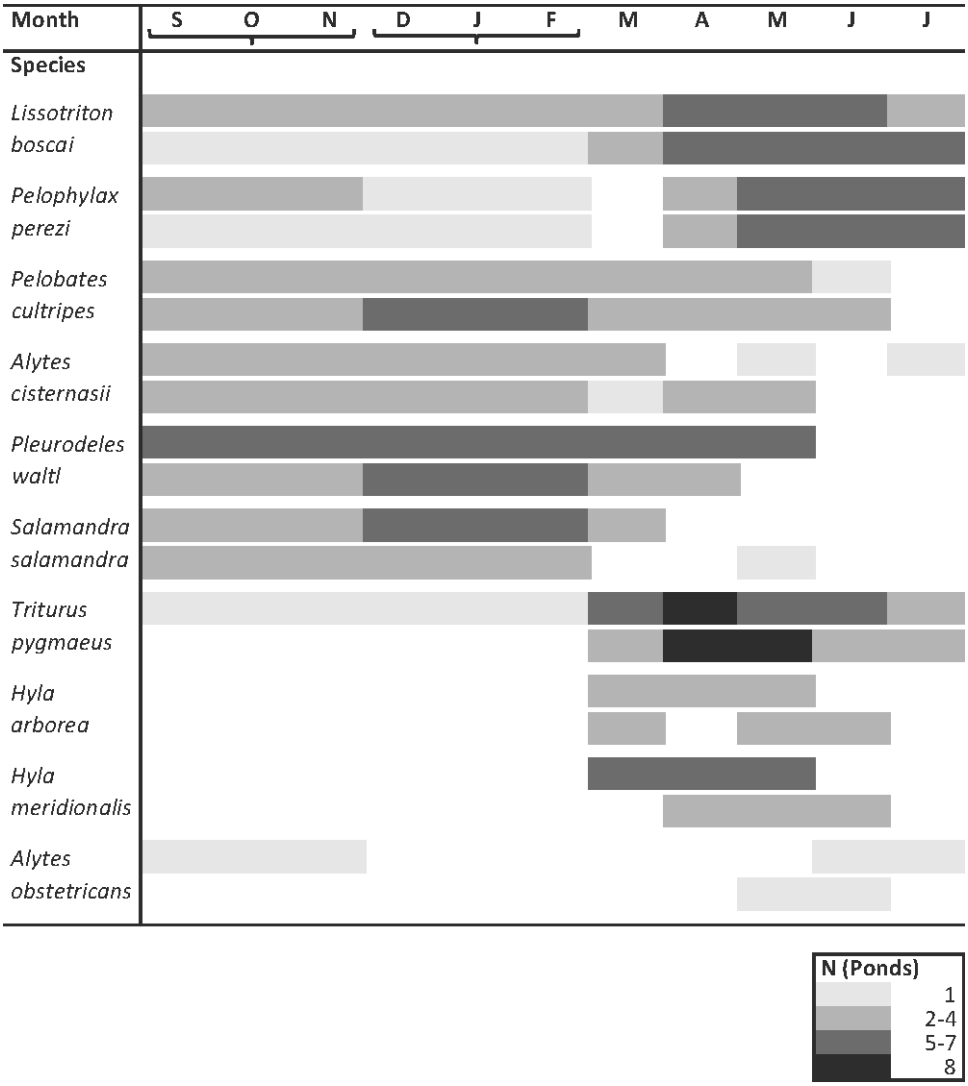

Figure 3. Phenology of each species from 2001 to 2003. Number of occupied ponds (larvae and eggs) per month. First bar: 2001/2002; second bar: 2002/2003. Data for autumn and for winter correspond to one month.

Species richness was negatively related to precipitation (P = 0.008, Table 2) and temperature (P = 0.002). Diversity of larvae was positively related to pond depth (P = 0.028). *Pleurodeles waltl* eggs were positively affected by precipitation (P = 0.003). *Salamandra salamandra* larvae showed a positive effect of precipitation (P = 0.009) and a negative effect of pond depth (P = 0.039). *Lissotriton boscai* larvae were negatively related to precipitation (P = 0.001), and larvae and eggs were positively related to pond depth (P = 0.006, P = 0.005). *Triturus pygmaeus* larvae were negatively related to precipitation (P = 0.001) and temperature (P = 0.040), and eggs were positively related to pond depth (P = 0.036). Moreover, the selected model for this species larvae retained pond depth with a positive effect, although not statistically significant. *Alytes obstetricans* larvae indicated a positive effect of temperature (P = 0.024). Finally, *P. perezi* larvae were positively related to temperature (P < 0.001) and eggs were negatively related to precipitation (P = 0.038).

Table 2. Regression coefficients of multiple linear regression models relating mean monthly species richness, mean monthly species diversity (Simpson's index), mean monthly overall abundance [log (CPUE + 1)], and mean monthly abundance per species [log (CPUE + 1)] to environmental variables: total monthly precipitation (mm), mean monthly temperature (°C), and mean monthly pond maximum depth (cm). Models selection based on AICc. P-values from *t* tests.

Assemblage / Species	Precipitation		Temperature		Max Depth	
	Estimate	P	Estimate	P	Estimate	P
Assemblage						
Species richness	-0.020	0.008	-0.249	0.002		
Diversity - Larvae					0.004	0.028
Diversity - Eggs						
Abundance - Larvae						
Abundance - Eggs						
Species - Larvae						
Pleurodeles waltl						
Salamandra salamandra	0.001	0.009			-0.001	0.039
Lissotriton boscai	-0.006	0.001			0.009	0.006
Triturus pygmaeus	-0.006	0.001	-0.039	0.040	0.005	0.052
Alytes cisternasii						
Alytes obstetricans			0.001	0.024		
Pelobates cultripes						
Hyla arborea						
Hyla meridionalis						
Pelophylax perezi			0.045	< 0.001		
Species - Eggs						
Pleurodeles waltl	0.003	0.003				
Lissotriton boscai					0.001	0.005
Triturus pygmaeus					0.006	0.036
Hyla arborea						
Hyla meridionalis						
Pelophylax perezi	-0.005	0.038				

Discussion

Our findings support the existence of an intra-annual pattern in amphibian larvae pond communities. Species richness and diversity, and overall abundance showed a seasonal pattern, with a peak in spring, supported by the fitted sinusoidal model. The community structure seasonal pattern resulted from each species exhibiting a circa-annual pattern, and the presence of different species phenological patterns. Moreover, phenological variability was also revealed between years and between ponds.

Our results show that ten of the fifteen species present in the region (Loureiro et al., 2008) bred consistently in the ponds, corroborating the importance of these temporary water reservoirs for amphibian reproduction (Oertli et al., 2002, 2005; Beja & Alcazar, 2003; Jakob et al., 2003; Vignoli et al., 2007; Gómez-Rodríguez et al., 2010a; Ferreira & Beja, 2013; Céréghino et al., 2014). Thus, five species were not recorded or were rare in the sampled ponds, although they were observed throughout the study. The larvae and eggs from three species were not collected in the ponds: *R. iberica* breeds typically in lotic waters, above 500 m in this region; *P. punctatus* breeds usually in flooded lands and small streams; and *D. galganoi* in flooded lands. For two other species, only larvae were recorded rarely: *B. bufo* breeds preferably in floodplains and slow running waters, and *E. calamita* in flooded lands and ephemeral ponds. *Salamandra salamandra* is ovoviviparous and *A. cisternasii* and *A. obstetricans* males carry the eggs until hatching, hence the eggs of these species are not observed in water bodies. Additionally, *P. cultripes* eggs were rarely collected, probably because this species is an explosive breeder and the embryonic development until hatching is fast. Finally, *A. obstetricans* larvae were recorded only seven times in the two ponds at highest altitudes, in agreement with the fact that in this region, which represents the southern limit of its distribution range, this species occupies medium to high altitude areas (Pargana et al., 1996; Arnold & Ovenden, 2002; García-París et al., 2004).

Species phenology

In our study each amphibian species exhibited a coherent phenological pattern among years and across ponds, and these patterns varied among species. Previous studies also found consistent and different breeding dates and larval periods for different species (Díaz-Paniagua, 1988, 1992; Jakob et al., 2003; Richter-Boix et al., 2006; Vignoli et al., 2007). Our results showed species that were present throughout the entire year, from autumn to spring, in autumn and spring/summer, from spring to summer, and only in spring.

There is also spatial variation in breeding and larval phenology within the species distribution ranges (García-París et al., 2004). Overall, the observed phenological patterns match, or are comprised within, the patterns found in other regions (García-París et al., 2004). The exception is *P. waltl*, observed from winter to summer in other regions, and exhibiting earlier breeding and larval periods in our study.

Our results overlap in general with those obtained by Pargana (1995) for the same region, a decade earlier (1993/1994), over a single year, and covering a wider variety of habitats. However, we observed *L. boscai* and *P. perezi* larvae over the entire year, while that study only found these species from spring to autumn. These two species had overwintering larvae, which was not referred by Pargana (1995), but has been found in other regions (García-París et al., 2004). Additionally, *P. cultripes* showed a longer larval period in our study, and *P. waltl* exhibited a much longer period, being present from autumn to spring in our study, but only in spring in 1994. Nevertheless, Pargana (1995) had few observations of *P. waltl*. *Alytes cisternasii* and *A. obstetricans* had two breeding periods, in autumn and in spring/summer, not identified by Pargana (1995), but described for other populations of *A. cisternasii* and coherent with the available information on *A. obstetricans* (García-

París et al., 2004). The differences observed can be related to the inter-annual variation of climatic conditions.

Species breeding dates and larval periods varied between years, in different degrees, and all species showed variability in the ponds occupied in the same month in different years. Spatially, species phenology varied between ponds, and most species were not recorded in at least one pond. Climate heterogeneity in time and space is characteristic of the Mediterranean region (Blondel & Aronson, 1999), and may cause plasticity in the reproductive strategies of the species, in response to unpredictability (Díaz-Paniagua, 1988, 1992; Jakob et al., 2003; Richter-Boix et al., 2006). Temporal and spatial phenological variability, as found in other studies (Oertli et al., 2002; Beja & Alcazar, 2003; Bosch & Martinez-Solano, 2003; Both et al., 2009; Gómez-Rodríguez et al., 2010a, 2010b), can be due to stochasticity, species plasticity, or local adaptation, related to variability in climate and local abiotic and biotic conditions (Wilbur, 1990; Wellborn et al., 1996; Griffiths, 1997). This can explain variability within this study as well as differences among studies, for the same region and for different regions (Pargana et al., 1996; García-París et al., 2004).

The response of species to environmental factors was roughly coherent with the previous knowledge on the species ecology (Arnold & Ovenden, 2002; García-París et al., 2004). *Lissotriton boscai* and *T. pygmaeus*, with breeding onset in spring, and larvae present all year in the first case, were dependent on water availability (pond depth) and negatively related to precipitation. *Pelophylax perezi*, whose breeding period and strong larvae presence occurred from spring to summer, was positively related to temperature and negatively related to precipitation. *Pleurodeles waltl* and *S. salamandra*, the two urodeles with breeding onset in autumn and larvae present until spring, were dependent on precipitation. Moreover, pond hydrology results partially from precipitation and temperature, which should be taken into account when considering the effects of these environmental factors on species ecological responses.

Community phenology

Our results show a circa-annual pattern for species richness, diversity of larvae and eggs, and overall abundance of larvae and eggs, peaking in the spring. The fitted sinusoidal model supports this clear community structure circa-annual pattern. So far, few studies have attempted to model community phenology periodicity. Canavero et al. (2008) applied a sinusoidal model to describe the pattern of anuran community calling activity. The present study now expands the application of these models to amphibian larvae communities.

This community circa-annual pattern results from the different species phenological patterns: *L. boscai* and *P. perezi* were present during the entire year; some species started breeding in the autumn and their larvae remained in the ponds until the spring (*P. waltl*, *S. salamandra*, *A. cisternasii*, and *P. cultripes*); other species bred in the spring and their larvae left the ponds in the spring (*H. arborea* and *H. meridionalis*) or in the summer (*T. pygmaeus*); and, finally, *A. obstetricans* was observed, rarely, in autumn and in spring/summer. Consequently, species richness was higher in spring, declined in summer, rose again in autumn, through winter, until the next spring. The same pattern was observed in species diversity and overall abundance. Therefore, winter does not seem to constrain the permanence of most larvae in the ponds. Furthermore, although *A. cisternasii* and *A. obstetricans* were bi-annual breeders, this did not affect the circa-annual general pattern, probably because they are only two (out of ten) species, which typically have small clutches.

As expected, there was a time lag between the egg and the larvae diversity sinusoidal waves, with the peak of the egg wave occurring earlier in the year than that of the larvae wave, and the same happening for the overall abundance. However, the time lag was smaller for diversity than for

abundance. The smaller time interval for diversity might be due to variation in development time. After each species starts breeding, it contributes to larvae diversity as soon as hatching begins. Some species with a shorter embryonic development period contribute sooner to the larvae diversity. Later, species with fast development metamorphose earlier, decreasing the larvae diversity. In contrast, overall abundance refers to the total number of eggs and larvae, and thus reflects the mean hatching time and mean development period of all species. Nevertheless, the larvae overall abundance results are less reliable, because its variation was less supported by the model.

The presence of intra-annual variation suggests that amphibian diversity surveys need to cover the entire year. Moreover, the inter-annual variability observed in our study region, and the high temporal fluctuations reported for other amphibian populations (Griffiths, 1997; Houlahan et al., 2000), indicate that long term studies are necessary in order to fully understand community patterns and processes. Additionally, a more frequent sampling protocol would contribute to detect a clearer pattern regarding the explosive breeders, namely *P. cultripes* (Arnold & Ovenden, 2002; García-París et al., 2004).

Pond depth, which is affected by precipitation and temperature patterns, had a peak in spring, and a pattern of variation that is consistent with the larvae diversity's positive relation with this environmental factor. Likewise, the pattern of species richness is consistent with its negative relation with temperature, which increased from winter to summer, as well as with its inverse relation with precipitation, which, although irregular, had minimums in summer and high values in autumn, winter, and spring. For most species the onset of breeding occurred in autumn and spring, corresponding to peaks of precipitation. Additionally, the phenological variability observed, between years and between ponds, may be related to the precipitation and hydrology variability registered, as referred by other studies (Wilbur, 1990; Díaz-Paniagua, 1992; Griffiths, 1997; Jakob et al., 2003; Richter-Boix et al., 2006). Summer appears to be the most limiting period for larval permanence in the ponds, with the majority of the species metamorphosing before or at the beginning of this period, as in other Mediterranean-type regions (Díaz-Paniagua, 1988, 1992; Jakob et al., 2003; Richter-Boix et al., 2006). Higher temperature and lower precipitation in the summer result in higher risk of desiccation. Thus, temperature appears to affect more strongly the termination of the larval period (Wilbur, 1990; Griffiths, 1997). These findings support the importance of long hydroperiod ponds, in addition to the maintenance of a network of ponds with naturally varying hydrology (Beja & Alcazar, 2003; Jakob et al., 2003; Vignoli et al., 2007; Gómez-Rodríguez et al., 2010b), for the conservation of amphibian diversity in the Mediterranean region.

Temporal segregation can contribute to species coexistence and thus to community structure and persistence (Wellborn et al., 1996; Céréghino et al., 2014). Our results show temporal partitioning among species. Other studies also reported temporal segregation as a driver of amphibian larvae community diversity. These studies suggest that temporal segregation reduces inter-specific interactions, competition for space and food, and may be related to different predation pressures in different periods. Temporal segregation in larvae may also be caused by temporal segregation in adult reproduction, due to competition, different breeding strategies and physiology (Díaz-Paniagua, 1988; Jakob et al., 2003; Richter-Boix et al., 2006; Vignoli et al., 2007).

Our study expands the application of sinusoidal models to amphibian larvae communities. These models allow the prediction of future trends in community dynamics, which can contribute to improve conservation management practices. Future research should focus on the development of these models and their coupling with environmental variables modeling.

Different species strategies, specifically breeding and larval period temporal segregation, may contribute to species coexistence, and hence to the maintenance of diverse communities (Díaz-Paniagua, 1988; Wellborn et al., 1996; Jakob et al., 2003; Richter-Boix et al., 2006; Vignoli et al., 2007). Moreover, phenology plasticity might favor the persistence of communities in unpredictable

environments as the Mediterranean region (Díaz-Paniagua, 1988, 1992; Wilbur, 1990; Jakob et al., 2003; Richter-Boix et al., 2006). However, because species richness and diversity, and most species phenologies are affected by climate and hydrology, climate change and manipulation of pond hydrology, namely pond regularization and leveling, may influence community structure and persistence (Griffiths, 1997; Beja & Alcazar, 2003; Ferreira & Beja, 2013; Walls et al., 2013; While & Uller, 2014; Benard, 2015; Lowe et al., 2015; Swan et al., 2015).

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